Revised Synopsis

Appendix – IV

Format For Outline Of Proposed Research Work

1) Name Of Scholar (In English) : Mrs Taslim Akhter

(In Hindi) : श्रीमती तस्लीम अख्तर

2) Title Of Research work

(In English) : A biochemical , haematological and histological study of effects of leaf extract of *Zizyphus nummularia* on Heart of Swiss albino mice exposed to Dimethoate.

(in Hindi) : 'डाइमेथोएट से परीक्षण करने पर स्विस एल्बिनो चूहे के हृदय पर *जिजाइफस नुम्मुलेरिया* के पर्ण निष्कर्षण के प्रभावों का जैवरासायनिक, रूधिर संबंधी एवं ऊतकीय अध्ययन'

3) Location : Department Of zoology

Govt. College , Ajmer (Raj)

Introduction and Importance of Proposed Investigation

From the beginning of life human has been constantly competing with the pest for food and shelter.Food production and disease control are the most important challenges facing humanity,that deserved special attention.

The reduced yield and Harvest looses are due to pest , by transmitting the diseases ,they are threatening human and live stock health. Due to this reason mostly research efforts are considered for controlling the pest and insect by using pesticides.

Pesticides are used for destroying,repelling or mitigating any pest not to affect human health.These are grouped under the basis of target organisms, chemical structure,physical state,biological mechanism ; but the most popular and preferable classification is based on the target organisms,means on which type of pest ,pesticides are being used.eg-Algaecide for Algae, Avicide for Birds, Bactericide for Bacteria, Fungicide for Fungi, Insecticide for Insect, Nematicide for Nematodes, Rodenticide for Virucide for Viruse etc.

Pesticides should have some ideal properties such as-

- It should be effective against pest.
- Lower stability in the environment.
- High toxicity for pest and low toxicity for plant,human and other warm blooded animals.
- It should be affordable.
- It should be non cumulative (no biomagnification).
- It should have acute and chronic effect.

Pesticides use raised the number of environmental concerns and are one of the major cause of-

- Water pollution
- Pesticides resistance
- Persistency in the environment is responsible for soil contamination.
- Biodiversity reduction
- Decline in pollinator

A particular quantity of insecticides causes side effect or toxic effect and mortal effect in living animals.These type of efforts are studied under the branch of

science"Toxicology" which is the science behind the stories, not always as dramatic but possibly as fascinating, and certainly necessary in determining how certain chemicals can harm us under particular conditions and seeking ways to prevent or alleviate the harm.

Dimethoate is the organophosphate is used extensively in horticulture as asystemic insecticide, is responsible for adverse effect on cardio-vascular, immune,nervous, hepatic, reproductive and respiratory system.

The most common names of Dimethoate are digon,duragon,rebelate and dimate.(Environmental Protection Agency,EPA,2006)

Zizyphus nummularia, also called Jharber in hindi, species of *Zizyphus* native to the Thar desert of western India and south eastern Pakistan and south Iran (where it is called ramilak in Persian)., belongs to the Rhamnaceae family, its synonym is *Zizyphus rotundifolia.*Ithas many medical importance such as-it is antihelminthic, antimicrobial, antidiabitic, Dried fruit used medicinally as astringent in bilious affliction in India.

The leaves are used to treat scabies and other skin diseases. The leaves are antipyretic and reduce obesity. The fruit is cooling, tonic, digestible and removes biliousness,thirst, vomiting and burning sensations . The dried fruits contain alkaloids, triterpenoids and saponins. They are anticancer, refrigerant, sedative, styptic, stomachic and tonic. They are used to purify the blood and aid digestion.

The proposed research work will deal with the effect of leaf extract of *Zizyphus nummularia* on the heart of dimethoate treated albino mice.This work will include histology of heart and its biochemical aspect as well as the haematological parameters of blood of dimethoate treated mice to ascertain and find out the persistence,accumulation and toxic effect of dimethoate, together with the effect of leaf extract of *Zizyphus nummularia* on dimethoate treated mice of different age group such as 70 and 105 days respectively, for the duration of 30 days.

Review of Literature/Work Already Done On the Subject of Proposed Research Work

Dautrman et al.(1959)examined that the dimethoate, which was orally administrated torat and lactating cows, its metabolites are found in blood, fat, faeces, urine and milk.In both species metabolism was rapid, with excretion occurring primarily in the urine.

Uchida et al.(1964) reported, a good correlation between the rate of metabolism of dimethoate and its toxicity towards the various species.

Hassan et al.(1969) administrated radioactive dimethoate via intraperitoneal injection to mice and found that, these radiolabelled metabolites were present in urine which were analysed by paper chromatography.Estimation was mostly in the urine (60%) during the first 24 hours.

Gunderson et al.(1988) reported the actual average daily intake of dimethoate by adults, is 0.008 µg/kg bw.

Hundley et al. (1988) analysed the domestic food sample and found that 96% of the food sample had level at or below 2 ppm.

WHO (1996) reported that, the extensive useof different pesticides in agriculture, has led to drastic effects in many non target species including man.

Kossmann et al.(1997) addressed the toxic effect of dimethoate on the functions of mammalian organs including liver and kidney and It was reported to alter the level of the marker parameters related to the liver and kidneys in rats.

Akingbemi et al.(2000) told that Endocrine toxicity of pesticides and reported their adverse effects on the reproductive axis of animal and humans.

Lotti(2001) reported dimethoate pesticides causeInhibition of brain acetyl cholinesterase (AChE) at synapses which results in, accumulation of acetylcholine and over activation of acetylcholine receptor at neuromuscular junction and in the autonomic and central nervous system.

Mahadevaswami and Kaliwal(2002) reported irragulatories of estrous cycle and altered level of serum gonadotropins in females ,due to dimethoate.

Budai et al. (2003) reported the teratogenic effects of a 38% dimethoate containing insecticide formulation in the chick embryo. Before the starting of incubation, the chicken eggs were directly exposed to the insecticide by injecting it. . There was no decrease in the embryonic body mass ,the rate of embryonic mortality was 31% and The developmental anomalies in surviving embryo occurred sporadically.

Mahadevaswami and Kaliwal(2004)studied developmental toxicity of dimetoate, which result in decreased number of implantation and live foetuses ,and decreased fetal body weights.

Mahjoubi-Samet et al. (2005) reported that Dimethoate can reduce thyroid secretory activity in suckling rats and may even decrease body growth. Thyroid hormone secretion at normal levels is essential for the growth and central nervous system maturation of suckling pups.

Costa (2006) toxicological profile of dimethoate demonstrates that it has anticholinesterase activity in all species including mice, rats and dogs.

Farag et al.(2007) reported that impairment of fertility, suppress libido, semen quality deterioration, altered testosterone level and testicular degeneration in males ,due to dimethoate.

Davies et al.(2008) Acute self-poisoning with the dimethoate pesticide has a human case fatality three-fold higher than poisoning with chlorpyrifos despite similar animal toxicity. Dimethoate poisoning is quite distinct from that of chlorpyrifos, many patients present with hypotension that progresses to shock and death within 12–48 h post-ingestion.

Singh et al. (2009) reported that carps are very sensitive to dimethoate as compared to air breathing teleost and they show behavioural changes which reflect its direct effect on nervous system of exposedfishes.

Srivastava et al. (2010) Found that the freshwater catfish, *H. fossilis*is more susceptible to dimethoate toxicity and its LC50 value is less than other reported fish species. This indicates that dimethoate exposure to the fish caused toxic effects. Further such fishes may affect the human being after consuming the fish as food.

Ganeshwade (2011) studied that a significant decrease in protein and glycogen content while an increase in ascorbic acid content in liver occurs in *puntius ticto* under dimethoate toxicity stress.

Kumar(2011)reported *Zizyphus nummularia is* used traditionally in treatment of mental retardation, preventing frequent attacks of influenza, colds, treating dysentery, diarrhoea and colic. It is also used in ulcers, fevers, wound healing, pharyngitis, bronchitis, burns, anaemia, irritability, hysteria and as a nervine tonic.

Gautam et al. (2011) studied that *Zizyphus nummularia* have potential antibacterial and antifungal activity against four medically important bacterial and fungal strains respectively *Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis, Pseudomonas aeruginosa, Aspergillus niger, Aspergillus flavus, Candida albicans* and *Trichophyton rubrum.*

Yusufoglu (2011) told that alcoholic extract of *Zizyphus nummularia* leaves formulated gel possessed anti inflammatory property,in comparison with marketed products,it showed a good wound healing effects ,can be an effective medication for inflammation and wound healing.

Rajasekaran et al.(2012)observed hypoglycemic and hypolipidemic effect of Ethanolic and aqueous extract of *Zizyphus nummularia* againstAlloxan-induced diabetic rats. Extracts showed significant anti-hyperglycemic and hypolipidemicactivity, reduces the elevated biochemical parameters and maintain body weight.

Ibtissem et al.(2012) reported that exposure to dimethoate cause oxidative stress in lung by an increase of malondialdehyde, protein carbonyl groups and advanced oxidation protein products. An increase in glutathione peroxidase, superoxide dismutase, catalase and a decrease in acetylcholinesterase and butyrylcholinesterase activities, glutathione, non-protein thiols and vitamins C levels.He found histopathological changes in lung tissue as emphysema, hemorrhages and hemosiderin deposits.

Yasin and Sharma(2013)reported thatdimethoate treated mice showed significant increase in RBCs, monocytes, basophills and neutrophills and a significant decrease in lymphocytes, haemoglobin count, PCV, MCV, MCH, MCHC and ESR.

Rajasekaran et al.(2013) told that hypoglycemic and hypolipidemic effect of Ethanolic and aqueous extract of *Zizyphus nummularia* against dexamethasone-induced diabetic rats. Extracts showed significant anti-hyperglycemic and hypolipidemic activity.

Goyal et al.(2013)studied that *ziziyphus nummularia* is an important ethno medicinal plant of the Thar Desert and local inhabitants use every part of the plant as medicine. Kernels are prescribed in pregnancy as soporific and for relieving abdominal pain.Crushed root is applied on the paining shoulder of the bullock. The decoction of leaves is used for the treatment of cough and cold; leaves are alsoregarded as diaphoretic and prescribed in

typhoid. Paste of leaves is used for healing of cuts, boils and cutaneous disease. It is widely used in pain and inflammatory conditions.

Penchalamma and Jacob (2014) reported kidney as the major detoxification organ for many xenobiotics, is frequently susceptible to their nephrotoxic effects. Acute renal failure was observed following exposure to dimethoate. The transient renal injury was attributed direct action of the dimethoate, causing tubular cell necrosis and to a secondary mechanism that followed the cholinergic crisis, causing hypovolemic shock and rhabdomyolysis.

Tanwar and Tejashree(2014)observed that dimethoate affects the normal functioning of the contractile vacuole and locomotor movements at the concentration range of 0.5 mg/ml and 1 mg/ml (and above) if exposed to the insecticide for 20 minutes and 3 hours respectively. It also affects the growth rate of the ciliate significantly and when subjected to the dimethoate (concentration 0.5mg/ml and above) for 24 hours and 96 hours exposure. Certain physiological deformities were also reported like irregular beating of cilia, blebbing and spinning movements.

Ngoula et al.(2014)studied testicularhistology of animals treated with high doses of dimethoate, showed sertoli cells destructionand disorganization of the germinal epithelium and the lumen of seminiferous tubules of treated mice wasgenerally poor in sperm.

Mohebbiet al.(2014) investigated *Zizyphus nummularia* kernel,fo r the removal of erythrosine toxin color from wastewater that causes irreparable damage on environmental cycle and human health. Erythrosine is known as severely pollutant to the aquatic environment and *Zizyphus nummularia* kernel had no significant application so it was suggested that the *Zizyphus nummularia* kernel may be used as a natural adsorbent of erythrosine dyes.

Penchalamma and Jacob (2015) reported that repeated oral doses of dimethoate exerts damaging effect on the cell metabolism which leads impaired protein synthesis machinery .It can be stated that long term exposure to dimethoate can result in cell metabolism toxicosis.

Daoud et al.(2015) found that Dimethoate induces genotoxic effects which invariably accompanied and correlated with oxidative stress.

Bahmani et al. (2015)studied thatmedicinal plant helpful in diseases and disorders and might be useful in gastrointestinal disorders. *Zizyphus nummularia* can be used to treat gastric disorders .

Kopaeieta11 .(2015) reported that *Zizyphus nummularia* leaf has traditional therapeutic effect on peptic ulcers.

Research Gap

Although a little work has been done on the toxicity of dimethoate on blood parameters and nervous system of wistar *rat*. Perusal of literature reveals that in the proposed area no work has been done on the parameters selected in the past decade of dimethoate effect on biochemistry, haematology and histology of swiss albino mice. Hence, the proposed work is aimed to observe the research gap along with the solutions of *Zizyphus nummularia* effect on the related problem.

Major hypothesis

The high toxicity level of dimethoate insecticide may disrupt the histology and biochemistry of heart of swiss albino rat. Long-term effects of dimethoate insecticide may results inhibition of cholinesterase ,atherosclerosis, heartattack,hypertension and stroke.

In the present study, we will study the protective effect of *Zizyphus nummularia* . The plant has been reported earlier.

Chemistry of Dimethoate insecticide

Chemical Group: Organophosphate

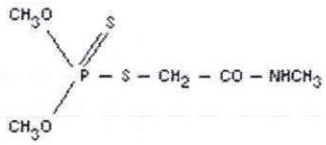
Synonyms-EI 12880, L 395, BAS 152, OMS 94, OMS 111, ENT 24 650, chemathoate, cygon, fosfamid, cekuthoate, daphene, devignon, dimet, dimethogen, trimetion.

Chemical names-*IUPAC O,O*-dimethyl *S*-methylcarbamoylmethylphosphorodithioate,2-dimethoxyphosphinothioylthio-*N*-methylacetamide*CA O,O*-dimethyl*S*-[2-(methylamino)-2-oxoethyl] phosphorodithioate

Molecular formula-

C5H12NO3PS2

Structural formula-



Relative molecular mass-

229.3 g/mol

History

It was first used in the 1960s after it was patented by American Cyanamid. It is moderately toxic to workers though there is little concern about accidental ingestion. Dimethoate was first described by Hoegberg and Cassaday in 1951 and introduced in market in 1956 (Fischer et al., 1997).Dimethoate is a widely used organophosphate insecticide, used to kill mites and aphids among other insects and is applied on citrus, cotton, fruit, olives, potatoes, tea, tobacco and vegetables. It is also used on flies in home gardens and on livestock.

Health Effects

The population as a whole is not generally subject to exposure to dimethoate from air, water or food; however occupational exposure may occur during manufacture, formulation and use. This mainly occurs through inhalation and dermal absorption, although occupational exposure can occur by accident or as a result of incorrect handling.

Acute Toxicity

Symptoms of acute poisoning of dimethoate are common with all other Organophosphates and revolve around cholinesterase inhibition. It is considered not as toxic as any other Organophosphates.

Chronic Toxicity

Chronic effects result in some of the same symptoms as the acute effects. There was not enough evidence of teratogenic, carcinogenic, or reproductive effects on humans to state anything definitely but it may have negative effects on all three of the categories listed above. Studies on mice and mice have shown certain negative reproductive and carcinogenic effects.

Environmental Effects

Dimethoate is highly mobile in the soil, labelled as a class II moderately toxic insecticide, somewhat persistent, and is not often found in large quantities in water . It is however, highly toxic to honeybees, moderately to highly toxic to birds, and moderately toxic to aquatic organisms. Maximam residue limit (pesticide residue) of dimethoate for seeds, fruits or berries, roots or rhizomes are 5mg/kg,0.5mg/kg,0.1mg/kg respectively.

Regulatory Status-

Dimethoate is a general use pesticide and it is a moderately toxic compound in EPA.

Objectives-

- To find out the persistent and toxicological effect of Dimethoate that is lethal to the swiss albino mice.
- To study the effect of doses of dimethoate such as-$1/5^{th}$ and $1/10^{th}$ of LD_{50} on different age group of swiss albino rat such as-70 and 105 days respectively.
- To study the biochemistry, haematology and histology of heart of Dimethoate treated mice.
- To study the biochemistry, haematology and histology of heart of Dimethoate treatedmice after administration of leaf extract of *Zizyphus nummularia.*

Expected duration of proposed research work

The proposed research work will be completed within three years.The year wise details are as follows-

Work to be carried out during 1st year

- Collection of literature particularly on methodology, review of literature and references.
- Standardization of protocol, including methodology.
- Pilot studies for setting up doses for experiment.

Work to be carried out during 2nd year - Experimental research work will be carried out.

To obtain the data, pertaining to fulfilment of the objectives set for the laboratory experiment will be carried out as given below-

- Study of toxicity of doses which are responsible for the physiological changes in mice.
- Study and analysis of biochemical and haematological changes, after treating with Dimethoate.
- Study and analysis of histological changes of target organ viz. heart, using modern techniques and tools.
- Study the effect of leaf extract of *Zizyphus nummularia* on Dimethoate treated rat by haematology and histology of heart.
- Statistical analysis of data obtained after treating with Dimethoate and leaf extract of *Zizyphus nummularia* respectively.

Work to be carried out during 3rd year-

- Compilation of data in comparative form.
- Preparation of hectographs (tables and figures etc.).
- Photomicrograph of histological analysis.
- Completion of data and publication of result in the form of research paper and presentation of entire research work in the form of thesis.
- Bibliography.
- Writing and Typing of thesis

Material and Method/ Research Methodology

The present study deals with the toxicological studies of Dimethoate insecticide and leaf extract of *Zizyphusnummularia*onswiss albino mice.

The proposed study plan is prepared as follows:

(A) Healthy albino mice of age group (70 days and 105 days of 52.65±2.41 and 61.09±2.89 grams respectively) will be taken for experimental study.

(B) The mice will be maintained in mice cages with stainless top grill, under laboratory condition

(C) All animals will be fed on standard diet and water adlibitum.

(D) Animals will be kept in laboratory condition .Treatment schedule will continue for 30 days. Oral Dose of Dimethoate will be 1/5 and 1/10 of LD 50. LD 50 is 150 mg/kg bw (WHO 2001).

(E) Oral dose of leaf extracts of *Zizyphusnummularia*250 mg/kg bw (Rajasekaran 2013) will be given to Dimethoate treated mice.

Preparation of extracts-

The fresh leaf of *Zizyphus nummularia* will be collected and dried under shade, sliced into small pieces and grounded into powder with mechanical grinder. Two hundred grams of powder was soaked in absolute ethanol and n-hexane solvent for 72 hr and then filtered through what man filter paper No. 1823. The process will be repeated three times. The filtrate will be evaporated through rotary evaporator to get the extracts and preserved in refrigerator at 4 ^{0}C.

Swiss albino mice will be divided into five groups of five mice each and treated as follows;

Group 1 Control

Group 2 Dimethoate (30 mg/kg.bw) day^{-1}

Group 3 Dimethoate (30 mg/kg.bw) day^{-1} and 60 min after *Zizyphus nummularia* leaf extract (250 mg/kg.bw day^{-1})

Group 4 Dimethoate(15 mg/kg.bw.) day^{-1}Dimethoate dissolved in distilled water

Group 5 Dimethoate (15 mg/kg.bw) day^{-1} and 60 min after *Zizyphus nummularia* leaf extract. (250 mg/kg.bw) day^{-1}

The effect of dose of Dimethoate on Heart will be studied after 30 days. Blood will be collected for haematological study and autopsy of the animal will be done, Heart will be dissected out for biochemical and histological studies of swiss albino mice.

A. Haematological Study-

1. Haemoglobin concentration-(Dacie and Lewis method,1991).
2. Haematocrit value- (Hawksley method 1981).
3. Erythrocyte Sedimentation Rate- (Dacie and Lewis, 1991).
4. RBC Count- (Dacie and Lewis,2001).
5. WBC Count -(Dacie and Lewis,2001).
6. Platelets Count-(Dacie and Lewis ,2001).
7. Packed Cell volume-(Dacie and Lewis,2001}.
8. MCV (Mean Corpuscular Volume).*
9. MCH (Mean Corpuscular Haemoglobin).*
10. MCHC (Mean Corpuscular Haemoglobin Concentration).*
11. Fibrinogen concentration- (clot weight method of Ingram, 1961).
12. Relative Plasma Viscosity- (Korubo-owiye et al., 1997).
13. Whole blood relative viscosity-(Korubo-owiye et al.,1997).
14. Prothrombin time- (Screening kits for thromboplastin and haemoscann).
15. Activated partial thromboplastin time-(Screening kits for thromboplastin and haemoscann).

*Red cell indices MCV, MCH and MCHC are calculated from haemoglobin, hematocrit, and red blood cell count.

B. Biochemical Study-

a) Lactate Dehydrogenase Enzyme-(Bruns and Bergmeyer's method,1965).

b) Acid Phosphatase Enzyme- (U.V. Kinetics method, 2008).

c) Alkaline Phosphatase Enzyme- (U.V. Kinetics method, 2008).

d) ATPase Enzyme- (Koch's method, 1970)

e) Cholesterol estimation -(Roeschlau's method, 1974)

C. Histological Study-

The mice Heart tissue intended for histological studies will be removed and immediately fixed in 10% phosphate buffered formalin for at least 24 hours. The tissue will be dehydrated in ethanol series (70%, 80%, 90%, and 100%), cleared in xylene and embedded in paraffin. 5 μm thick sections were taken using a rotary microtone and sections will be affixed on clean glass slides by gentle heating. The slides will be deparaffinised and stained with haemetoxylins and eosin and they will be mounted with a cover slip using DPX. The slides will be observed under a light microscope for histological study.

Facilities Available For The research work-

All the research facilities(laboratory space,instrument and literature)required for the research work will be provided by Head of the Department of Zoology, Govt. College,Ajmer(Raj.) and by J.L.N.medical college,Ajmer,(Raj.)

Instruments for the scheduled study are as follows-

1. Computer: Various software which are required for complex analysis etc.
2. Electron Microscope
3. Gas Chromatography
4. Gavage
5. Kits for biochemical and haematological estimation
6. Light Microscope
7. Matched Cuvettes
8. Mechanical grinder

9. Microtome
10. Oven
11. Rotary Evaporator
12. Slides
13. Waxes
14. What'sman filter paper no.1823

Chapter Wise Detail of Proposed Research Investigation

The thesis will be presented in the following chapters which would give concrete form to it.

Chapter I – Introduction and objectives

The chapter will comprise the need of research work on the animal swiss albino mice and the significance and pertinence of the work. A frame will be presented with the clear picture of well defined objectives.

Chapter II –Review Of Literature

The chapter will include the reports and findings done so far at national and international level by various workers in the field of haematology and histology of heart affected by dimethoate and leaf extract of *Zizyphus nummularia.*

Chapter III-Study Area and Parameter

The chapter will contain the detail layout of insecticide under investigation, its natural occurrence, persistence, brief history, toxicity and regular environmental status as well as other parameters which were covered in the heading of various modern techniques.

Chapter IV-Research Methodology

This chapter will consist of various methods and techniques pertinent to haematological and histological studies with respect to toxicity of insecticide (Dimethoate) on heart of swiss albino mice and effect of leaf extract of *Zizyphus nummularia* on dimethoate treated mice. Laboratory analysis will be done and methodology would be mentioned in this chapter.

Chapter V-Observations

The chapter will include the data collected during the study of the effect of leaf extract of *Zizyphus nummularia* against the toxicity of insecticide (Dimethoate) on swiss albino mice in which we will include haematology and histology of heart.

Chapter VI-Result and Discussion

This chapter will include the effect of leaf extract of *Zizyphus nummularia* against the toxicity of insecticide (Dimethoate) on swiss albino mice, in order to protect mammals and other animals. The observation made during the study period would be deeply discussed,interpreted and compared with reports available on other animals.

Chapter VII-Conclusion

This chapter will contain consequences and important findings of research suggestions for protecting mammal and other animals from the adverse and mortal effect of Dimethoate and the effects of leaf extract of *Zizyphus nummularia* against the toxicity of insecticide (Dimethoate).A clear picture will be drawn by Showing toxicological effects on swiss albino mice because their anatomy and physiology is very similar with human anatomy and physiology, ther efore to protect the human being from adverse effect of Dimethoate which is commonly used in agriculture, this research work would be an important scientific contribution in the field of toxicology and beneficial for the society.

Literature Cited

It will include a list of references used in the text and also those included in the Appendix, and Website references.

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Signature of the Candidate

Revised Outline Approved

प्राणी शास्त्र विभाग
राजकीय महाविद्यालय, अजमेर
Dr Sabiha Khan 25/5/15

Name and signature of the Supervisor

With date and seal

APPENDIX - VI

MAHARSHI DAYANAND SARASWATI UNIVERSITY, AJMER

Certificate By Supervisor

Certified that :

1. I am willing to guide the research work of Shri/Km./Smt. Taslim Akhter on the above subject if he/she is registered for Ph.D.
2. To the best of my knowledge, the subject selected has not been studied and is not being studied so far in any University.
3. The subject is of sufficient scope to keep the candidate engaged for two years.
4. The subject will lead to a valuable contribution. I have seen and approved the outline of proposed research work submitted by the candidate.
5. I have ..01.. (give numbers) Research candidates registered under my supervision and the serial number of this candidate whose application is being forwarded for registration is ..02.. (give the serial number)
6. The candidate is not in a close relation (the term of close relation is defined in the examination ordinance of the University)

प्राणी शास्त्र विभाग
राजकीय महाविद्यालय, अजमेर

25/5/15

Signature of the Supervisor with date

Certified that the available facilities will be extended to Shri/Km./Smt. ..... for research work if he/she is registered for Ph.D.

उप-प्राचार्य

Signature of the Principal/ Director of the Institution with date

प्रभारी
प्राणीशास्त्र विभाग,
राजकीय महाविद्यालय, अजमेर

25/5/15

Signature of the Head, Department with date